

DOP-PCR Labeling (SKY)

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

Agarose, Ultrapure

Gibco BRL, Cat. 15510-027

Ampli Taq polymerase 5 U/μl

Perkin Elmer, Part No. N808-0158

Buffer, 10X and 25 mM MgCl₂

Perkin Elmer, Part No. N808-00

Loading buffer, 5X

Quality Biological, Cat. 51-026-030

Ethidium Bromide

Research Genetics, Cat. 750007

Primer "UN1"

Midland Certified Reagent Co. Telenius 6MW
[5'-CCGACTCGAGNNNNNNATGTGG-3']

10X TAE buffer

Advanced Biotechnologies, Cat. 08-514-001

Template DNA

Use 400 – 600 ng for each reaction

100 mM dNTP nucleotides

dGTP

Boehringer Mannheim, Cat. 1051466

dCTP

Boehringer Mannheim, Cat. 1051458

dATP

Boehringer Mannheim, Cat. 1051440

dTTP

Boehringer Mannheim, Cat. 1051482

x – dUTP:

Spectrum Green

Vysis, Cat. 30-803200

Spectrum orange (1 mM)

Vysis, Cat. 30-803000

Texas red (1 mM)

Molecular Probes, Cat. C-7631

Biotin (50 mM)

Boehringer Mannheim, Cat. 1093070

Digoxigenin (50 mM)

Boehringer Mannheim, Cat. 1558706

Water, sterile (H₂O)

Molecular grade sterile distilled water

Materials and Equipment

PCR Thermocycler

MJ – Research, Inc., Model PTC - 100

Gel system and power source

PCR tubes

PGC Scientifics, Cat. 502-075

Preparation

1X TAE buffer

Dilute 10X TAE with dH₂O

1% agarose gel

Dilute 1 g of agarose into 100 ml of 1X TAE buffer, heat until dissolved

Stock dNTP 2mM

	<u>μl</u>	<u>mM final</u>
dGTP	10	0.2
dCTP	10	0.2
dATP	10	0.2
dTTP	7.5	0.15
dH ₂ O	<u>462.5</u>	-
Total	500	

x – dUTP's

Spectrum Orange (SpO) is diluted 1:5 in sterile H₂O (14 μl SpO + 56 μl H₂O).

Texas Red (TR) is diluted 1:2 in sterile H₂O (30 μl TR + 30 μl H₂O).

Spectrum Green (SG) is diluted 1:5 in sterile H₂O (13 μl SG + 52 μl H₂O)

Microcentrifuge tubes, pipette tips, and pipettes

Autoclave PCR microcentrifuge tubes (0.5 ml size), 2 ml microcentrifuge tubes, and pipette tips (10, 200, and 1000 μl).

Sterilize pipettes (using UV) to be used for PCR and only use that set for PCR (can use Stratalinker or UV light source from TC hood).

Workspace

Before starting make sure that the work space is cleaned with ethanol and there is a steady air flow; if not, then use the tissue culture hood to maintain sterility.

Template DNA

400 – 600 ng (see note 1)

Procedure

Use sterile techniques throughout, especially when handling the DNA samples.

1. Label PCR tubes according to labeling scheme (note 2) (e.g. 1A, 1E, etc.) plus a zero control for each color (master mix with no DNA sample).
2. Defrost template DNA at 37°C, vortex and spin briefly prior to aliquoting out the DNA. Aliquot 4 µl of the appropriate DNA into each PCR tube, seal lid, and temporarily place at 4°C.
3. Label five 2 ml microcentrifuge tubes (A – E) and make the master mix for each fluorochrome according to the table (table 1) below and put on ice. The order for pipetting the master mix is as follows: dH₂O, buffer, MgCl₂, dNTP, and primer.
4. Dilute the TR, SpO, and SG dUTP's immediately before use. Vortex the labeled fluor dUTP's, spin down briefly and add each to the appropriate master mix and place back on ice.
5. Mix the Taq enzyme carefully (tap with finger), spin down, then add appropriate amounts to each master mix. Vortex the master mix tubes, spin and put back on ice.
6. Put PCR tubes with aliquoted DNA in fluorochrome order (all A's in one row, all B's in the next, etc.)
7. Carefully open each tube in row A by handling only the outside of the tube. Vortex the master mix for SpO, pipet 96 µl into each tube (change tips between each tube) and put on ice. Repeat the same procedure for each remaining x-dUTP.
8. Vortex each tube, spin down briefly and put them on ice again.
9. Start the PCR machine and select the PCR program (note 3), arrange the tubes in the PCR machine, close lid, and start the program.
10. After completion, remove the tubes from the PCR machine, vortex, spin down, and place on ice. In a round bottom Elisa plate, place 0.8 µl of 5X

DNA loading buffer in each well to which you will be adding a 2 μ l aliquot from each reaction to run on a 1% agarose gel. The resulting smear migrates to around 500 bp (note 4).

Table 1.

	Spec Orange A	Texas Red B	Bio (Cy5) C	SG D	DIG (Cy5.5) E
10X PCR buffer	130	120	140	150	130
MgCl ₂ (25 mM)	104	96	112	120	104
1-dNT (2 mM)	65	60	70	75	65
sterile dH ₂ O	819	756	910	945	845
Primer (100 μ M)	52	48	56	60	52
PE AmpliTaq polymerase	13	12	14	15	13
α -dUTP	65 (dil.1:5)	60 (dil.1:5)	42	75	39
No.reactions	1248:96=13	1152:96=12	1344:96=14	1440:96=15	1248:96=13

Notes

1. The stock DNA used for labeling PCR reactions is initially amplified as described in the primary DOP-PCR protocol. This amplified product can then be amplified again as described in the secondary DOP-PCR protocol. It is this secondary amplification product that is then used as the starting material for the SKY labeling PCR reactions.
2. Labeling scheme:
 A-Spectrum Orange
 B-Texas Red
 C-Biotin
 D-Spectrum Green
 E-Digoxigenin

 chromosome 1-AE
 2-E
 3-CDE
 4-BC
 5-ABDE
 6-BE

7-CD
 8-D
 9-ADE
 10-CE
 11-A
 12-BCDE
 13-AD
 14-B
 15-ABC
 16-BCD
 17-C
 18-ABD
 19-AC
 20-ACD
 21-DE
 22-ABCE
 X-BD
 Y-ACDE

3. PCR program :

Step	Temperature (° C)	Minutes
1	94	1
2	56	1
3	72	3 with addition of 1 sec/cycle
4	repeat steps 1-3, 29 times	
5	72	10
6	4	∞

4. The 1% agarose gels migrate to about 500 bp.

